Kidney International, Vol. 59 (2001), pp. 2233–2242

Renal phenotype of low kallikrein rats

Paolo Madeddu, Carlos P. Vio, Stefania Straino, Maria Bonaria Salis, Anna Franca Milia, and Costanza Emanueli

National Laboratory of the National Institute of Biostructures and Biosystems, Osilo, and Department of Internal Medicine, University of Sassari, Sassari, Italy; Chatolica Universidad, Santiago, Chile; and Laboratorio di Patologia Vascolare, Istituto Dermopatico dell'Immacolata (IDI-IRCCS), Roma, Italy

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Background. Renal kallikrein has been linked with inheritance of arterial hypertension and with sensitivity to drug nephrotoxicity. Identification of a cause–effect relationship between low kallikrein and intermediate phenotypes has been hampered by the lack of adequate animal models.

Methods. Kallikrein was measured in tissues obtained from rats inbred for low urinary kallikrein excretion (LKR) and wild-type controls. Blood pressure and indices of myocardial contractility were recorded via an intraventricular cannula connected to a transducer. The functional relevance of endogenous angiotensin II (Ang II) in LKR was explored by determining the effect of Ang II subtype 1 (AT_1) receptor blockade on glomerular filtration rate, renal blood flow, and urinary sodium excretion. In addition, sensitivity to gentamycin-induced nephrotoxicity was evaluated.

Results. Kallikrein activity was reduced by 60% in the kidney of LKR ($P < 0.01$), whereas it was increased in the heart ($P < 0.05$) and was unaltered in the pancreas, liver, and salivary glands. Heart rate and myocardial contractility were reduced, and the mean blood pressure (MBP) was increased in LKR as compared with controls ($P < 0.05$). LKR exhibited polydipsia, polyuria, glomerular hyperfiltration, and reduced fractional sodium excretion under basal conditions and impaired renal vasodilation in response to volume expansion. These functional alterations were significantly attenuated by AT_1 receptor blockade. Gentamycin reduced the glomerular filtration rate in LKR, but not in controls.

Conclusions. In LKR, unopposed activity of Ang II appears to be responsible for increased glomerular hydrostatic pressure and augmented tubular reabsorption. Balance between the kallikrein-kinin and renin-angiotensin systems is essential for normal renal function.

The serine proteinase tissue kallikrein (EC 3.4.21.35) cleaves low molecular weight kininogen to release the vasoactive kinin peptide [1]. Activation of G-protein–coupled kinin B_1 and B_2 receptors mediates a broad spectrum of biological effects, including renal vasodilation, diuresis, and natriuresis [2, 3]. In the Utah study, urinary kallikrein was found to be primarily influenced by genetic factors, with 51% of the total variance being caused by a single gene [4]. Reduced urinary kallikrein excretion was reported in genetically hypertensive rats, and cosegregation of a kallikrein gene polymorphism with high blood pressure was recognized by crossing spontaneously hypertensive (SHR) with normotensive Brown Norway rats [5–7]. Low urinary kallikrein phenotype is regarded as an indicator of blood pressure sensitivity to salt in humans [8]. Susceptibility to develop hypertension with an introduction of high salt intake was documented in knockout mice lacking the bradykinin B_2 receptor gene [9, 10], thus suggesting that a defective kallikrein-kinin system may sensitize these animals to the deleterious cardiovascular effects of high sodium.

Besides being genetically determined, the kallikrein phenotype appears to be influenced by common environmental factors, as suggested by a strong spouse–spouse correlation. Dietary potassium was indicated as the major factor responsible for noninherited variance [11, 12]. This interaction may be instrumental to the antihypertensive action of dietary potassium supplementation in essential hypertensives [13–15] or young SHR [16]. Recently, the beneficial properties of kallikrein have been extended to include renal protection and repair. In fact, administration of tissue kallikrein in the form of purified protein or by the way of adenovirus-mediated gene transfer reportedly exerts renal protection in Dahl salt-sensitive rats, two-kidney, one-clip Goldblatt hypertensive rats, and gentamycin nephrotoxic rats [17–19].

In the past years, a strain was developed in our laboratory by breeding rats from a Wistar stock according to low urinary kallikrein levels, exclusively [20]. Low-kallikrein rats (LKRs) develop mild-to-moderate blood pressure elevation when exposed to high dietary sodium intake. Histologic abnormalities were detected at different levels of the nephron, functionally related to the altered renal phenotype. These alterations did not preclude cor-

Key words: blood pressure, glomerular filtration rate, renal blood flow, kinins, arterial hypertension.

Received for publication August 9, 2000
and in revised form December 22, 2000
Accepted for publication January 8, 2001

rection of salt sensitivity by exogenous kallikrein supplementation [21, 22]. LKR may offer a unique opportunity for studying the influence of environmental factors on renal function in the context of an inherited defect in renal kallikrein expression.

The present study focused on characterizing the cardiac and renal phenotype of LKR and determining whether defective expression is restricted to the kidney. We also evaluated whether functional abnormalities typical of this strain can be corrected by blockade of angiotensin II subtype 1 (AT_1) receptors. Finally, whether low kallikrein sensitizes to gentamycin-induced nephrotoxicity was evaluated.

METHODS

Male rats were housed at a constant room temperature (24 ± 1°C) and humidity (60 ± 3%) with a 12-hour light/dark cycle. They had free access to rat chow (sodium, 0.12 mmol/g, Mucedola, Settimo Milanese, Italy) and tap water, except in selected sets of experiments. All procedures complied with the standard for the care and use of animals, as stated in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy of Science, Bethesda, MD, USA).

Immunohistochemical localization of renal kallikrein

Anesthetized Wistar rats and LKRs (N = 5 and 6, respectively) were perfused at physiologic pressure with phosphate-buffered saline (PBS), followed by buffered paraformaldehyde (pH 7.4). Kidneys were harvested and fixed in buffered paraformaldehyde for 24 hours at room temperature. The samples were then dehydrated in ethanol and embedded in paraffin. Renal sections (5 μm) were cut and incubated overnight at 22°C with an antibody raised against rat tissue kallikrein [23]. At variance with the antiserum used in a previous study [22], this antibody does not cross-react with esterase A. Then, a second antibody (swine anti-rabbit IgG; Dako, Carpinteria, CA, USA) at a dilution of 1:80, and peroxidase/antiperoxidase complex (Dako) at 1:100 was applied for 30 minutes each. The peroxidase activity was visualized by incubating the sections in a 0.1 (wt/vol) of 3,3′-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) and 0.03% (vol/vol) H_2O_2 solution for 15 minutes in the dark. Controls were prepared by omission of the first antibody and replacement by nonimmune rabbit serum at the same dilutions. Three sections were examined for each animal. Tubular cells were counted in each field, using a square ocular grid, to compute cell density.

Left ventricle performance and kallikrein activity in various tissues

Wistar rats and LKRs (3 or 6 months of age, N = 6 each group) were anesthetized with an intramuscular injection of ketamine (20 mg/kg body weight) and medetomidine chloridrate (0.4 mg/kg body weight) and were kept at 37°C on a heating pad. Left intraventricular blood pressure and heart rate (HR) were measured with a high precision transducer (World Precision Instruments, Basile, Comerio, Italy) via a G22 needle inserted through the chest wall [24]. Data were collected on a four-channel PowerMcLab polygraph and stored for subsequent computation of left ventricular end diastolic pressure (LVEDP) and maximal change in developed pressure (dP/dt_{max}). At the end of measurements, blood was sampled for determination of hematocrit. The animals were then perfused with PBS at physiologic pressure via the intraventricular needle. The heart, kidneys, submandibulary glands, liver, and pancreas were harvested for determination of kallikrein activity in homogenates.

Gentamycin-induced nephrotoxicity

Wistar rats and LKRs were randomly divided into four groups (N = 6 each). The first one was injected with sterile saline (2 μL/g body weight, subcutaneously). The other three groups received gentamycin sulfate (Sigma-Aldrich) at the doses of 20, 40, or 80 mg/kg body weight in saline for ten consecutive days. Twenty-four–hour urine collections were obtained on day 10, with the rats deprived of food to avoid urine contamination. Urinary volume (UV) was calculated gravimetrically, and samples were used for analysis of urinary sodium (U_{Na}), kallikrein (U_{Kall}), creatinine (U_{Cr}), protein (U_{Prot}), and N-acetyl-β-D-glucosaminidase (U_{NAG}). In addition, blood was sampled via a small incision of the tail for determination of plasma creatinine (P_{Cr}). The animals were then weighed and sacrificed. Both kidneys and the heart were harvested, cleaned, washed in saline, blotted, weighed, fixed in buffered formaldehyde solution, and paraffin embedded. Kidney sections, stained with hematoxylin and eosin, were analyzed using light microscopy.

Characterization of renal function under basal conditions and following AT_1 receptor blockade or volume expansion

Blood (0.5 mL) was obtained from unanesthetized rats of each strain (N = 6 per group) through an incision of the tail for measurement of plasma renin activity (PRA). The following day, measurements of urine flow rate, glomerular filtration rate (GFR), and renal blood flow (RBF) were performed with animals anesthetized and placed on a heating pad at 37°C [19]. Infusion of inulin and para-aminohippuric acid (PAH) was performed via a cannula inserted into the left femoral vein, while measurement of blood pressure and a blood sampling were made via a catheter inserted into the left femoral artery. The bladder was cannulated for urine collection. Forty-five minutes were allowed for the preparation to reach a steady state. Two timed urine collections were obtained, with blood (0.6 mL) collected at the end of each clearance period. GFR and renal plasma flow were deter-

mined from the clearance of inulin and PAH, respectively, and were normalized to kidney weight.

In separate experiments, 24-hour urine collections were obtained, as specified previously in this article. At the end of collection, the animals were allowed to return to their cages with free access to food. The following day, rats were intraperitoneally injected with the nonpeptidic AT_1 antagonist A-81988 [2-(N-propyl-N-2′(1H-tetrazol-5yl)biphanyl-4-yl)methyl amino pyridine-3-carboxylic acid] at 1.7 mg/kg body weight in 100 μL sterile saline [25]. One hour later, the animals were placed in metabolic cages for urine collection. Blood was withdrawn at the end of this 24-hour collection according to the procedure indicated previously in this article.

In additional studies, rats ($N = 10$ per group) were anesthetized and instrumented with a jugular catheter for drug infusion and a femoral artery catheter for continuous measurement of mean blood pressure (MBP) using a transducer connected to a Power McLab polygraph. In addition, a Doppler flowmeter probe was positioned on the left renal artery through an incision of the abdominal wall for measurement of red cell velocity, an index of renal blood flow (RBF) [26]. After a ten-minute stabilization period, the AT_1 antagonist A-81988 (5 mg/kg body weight in 100 μL saline) was injected intravenously, and hemodynamic parameters were recorded for an additional 10 minutes.

To test the effects of acute volume expansion, anesthetized rats ($N = 7$ per group) were infused with saline (5% body weight) intravenously over three minutes [27]. MBP and Doppler RBF were continuously recorded under basal conditions and for 30 minutes following saline.

Concentration test

In Wistar rats and LKRs ($N = 5$ per group), concentration ability was evaluated by determining UV and osmolarity (U_{Osm}) before and after water deprivation. To this aim, 24-hour urine collections were obtained under basal conditions and then starting 18 hours after water withdrawal.

Biochemical assays

Plasma renin activity was measured by radioimmunoassay. Inulin and PAH concentrations were determined by anthrone and colorimetric methods, respectively. Urinary kallikrein activity was measured in urine and tissue homogenates by an amidolytic assay, as previously described [20]. U_{NAG} levels were determined using a colorimetric assay [20]. Sodium concentration was determined by flame photometry. P_{Cr}, U_{Cr}, and U_{Osm} were measured with an autoanalyzer.

Statistical analysis

All results are expressed as mean ± SEM. Multivariate repeated-measures analysis of variance (ANOVA) was performed to test for interaction between time and grouping factor. In multiple comparisons among independent groups in which ANOVA and F test indicated significant differences, the statistical value was determined according to the Bonferroni's method. Differences within or between groups were determined using the paired or unpaired Student t test, respectively. A value of $P < 0.05$ was interpreted to denote statistical significance.

RESULTS

Cardiovascular phenotype of LKR

As indicated in Table 1, LKRs showed reduced body weight gain from three to six months of age compared with controls ($P < 0.05$). Heart weight normalized by body weight was higher in LKR at three months, but not at six months.

The HR of anesthetized LKR was significantly lower at both ages examined as compared with wild-type controls, this bradycardia being associated with reduced left ventricular contractility index, dP/dt_{max} ($P < 0.05$ for both comparisons). Systolic blood pressure and LVEDP did not differ between strains. In contrast, MBP was modestly but significantly increased in LKR (82 ± 2 vs. 71 ± 3 mm Hg in controls at 3 months, $P < 0.05$).

Kallikrein activity in tissue homogenates

As shown in Figure 1, in three-month-old LKR, renal kallikrein activity was reduced by 60% compared with controls ($P < 0.01$). In the same animals, kallikrein was increased in the heart ($P < 0.05$) and was unchanged in the pancreas, liver, and submandibulary glands (P = NS).

Immunohistochemical localization of renal kallikrein

Kallikrein immunoreactivity was restricted to connecting tubules without any staining in proximal or other cortical or medullary tubules. As shown in Figure 2, there was a striking difference between strains. In LKR, faint staining was observed, with kallikrein mostly located at the periphery of the connecting tubular cells. Kallikrein-positive cells were reduced in terms of density or percentage of total cortical tubular cells (175 ± 9 vs. 386 ± 26 cells per 0.16 μm^2 in controls and 9.6 ± 2.9 vs. 20.7 ± 1.2% in controls, $P < 0.01$ for both comparisons), while no group difference was detected in tubular cell size (112 ± 2 vs. 111 ± 6 μm^2, P = NS). In addition, LKR exhibited tubule lumen dilation.

Gentamycin nephrotoxicity

As shown in Table 2, vehicle-treated LKR showed elevated UV and GFR and reduced fractional sodium excretion (FE_{Na}) compared with controls ($P < 0.05$). Gentamycin dose dependently reduced GFR in LKR ($P < 0.05$) only. The latter effect was associated with a significant increase in FE_{Na}.

Table 1. Hemodynamic characteristics of low kallikrein rats (LKR)

	Wistar rats		LKR	
Age *months*	3	6	3	6
Body weight *g*	286 ± 12	488 ± 30	321 ± 8	419 ± 9[a]
Heart wt *g/100 g body weight*	0.27 ± 0.01	0.24 ± 0.01	0.30 ± 0.01[a]	0.26 ± 0.01
SBP *mm Hg*	118 ± 3	120 ± 2	119 ± 3	122 ± 2
MBP *mm Hg*	71 ± 3	72 ± 3	82 ± 2[a]	84 ± 2[a]
HR *beats/min*	263 ± 10	232 ± 20	199 ± 9[a]	173 ± 18[a]
dP/dt_{max} *mm Hg/sec*	5396 ± 152	6001 ± 229	4070 ± 392[a]	3952 ± 674[a]
LVEDP *mm Hg*	7.2 ± 0.7	6.3 ± 2.8	6.4 ± 1.7	8.3 ± 2.0

Values are mean ± SEM. Abbreviations are: SBP, systolic blood pressure; MBP, mean blood pressure; HR, heart rate; dP/dt_{max}, maximal change in developed pressure; LVEDP, left ventricular end diastolic pressure.
[a] $P < 0.05$ vs. Wistar rats of the same age

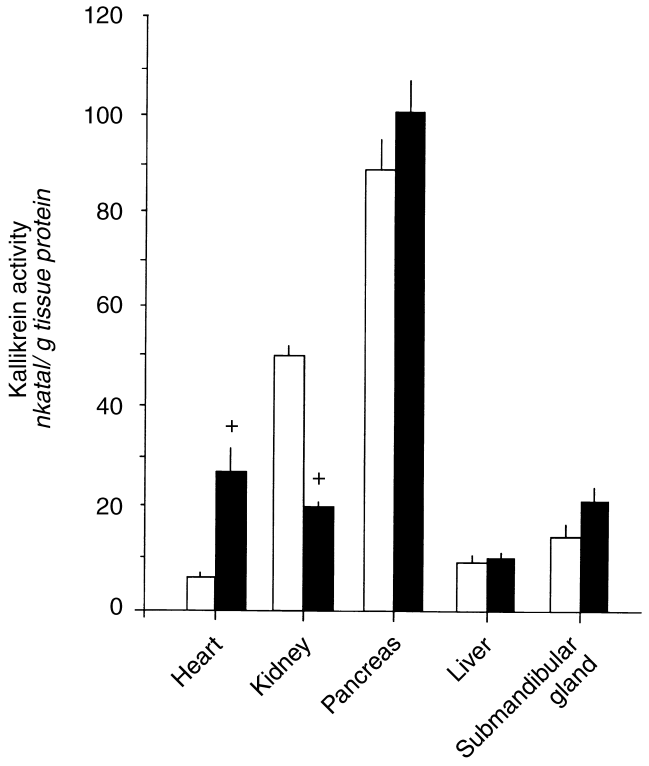


Fig. 1. Kallikrein activity in tissue homogenates of normal Wistar rats (□) and low kallikrein rats (LKR; ■). Kallikrein activity was reduced in kidney and was increased in heart of LKR. Data are means ± SEM ($N = 6$). $^{+}P < 0.05$ vs. Wistar rats.

The $U_{Kall}V$ was not affected by vehicle, whereas it was dose dependently decreased by gentamycin in both strains ($P < 0.05$; Table 2 and Fig. 3), with LKRs showing lower kallikrein levels for each dose tested ($P < 0.01$).

The $U_{NAG}V$ was elevated in vehicle-treated LKRs compared with controls ($P < 0.05$). Following gentamycin, enzymuria was similarly augmented in both strains. The highest dose of gentamycin increased $U_{Prot}V$ (from 12 ± 5 to 42 ± 3 mg/day in LKR and from 10 ± 6 to 41 ± 4 mg/day in controls, $P < 0.05$ for both comparisons).

No strain-related difference was observed regarding kidney weight or kidney-to-body weight ratio within vehicle treatment. Gentamycin treatment resulted in similar increases in kidney weight in both strains. Renal sections of gentamycin-treated rats showed histologic alterations, mainly consisting of tubular dilation and damage with necrotic cells filling proximal tubule lumen (data not shown). These morphologic alterations were quantitatively similar in LKRs and controls.

The heart-to-body weight ratio was higher ($P < 0.05$) in vehicle-treated LKRs (0.32 ± 0.02 g/100 g body weight) as compared with controls (0.25 ± 0.01 g/100 g body weight), and this difference was maintained in rats given gentamycin (0.28 ± 0.05 vs. 0.24 ± 0.01 g/100 g body weight in controls, $P < 0.05$).

Characterization of renal function under basal conditions and following AT_1 receptor blockade or volume expansion

Functional studies performed with rats under the effects of anesthesia showed significantly higher inulin clearance in LKR (0.42 ± 0.05 vs. 0.33 ± 0.03 mL/min/g kidney weight in controls, $P < 0.05$), whereas the clearance of PAH did not differ between groups (data not shown).

Plasma renin activity was significantly lower ($P < 0.05$) in unanesthetized LKRs (10.75 ± 4.28 ng/mL/h) as compared with controls (27.84 ± 3.89 ng/mL/h). Pharmacological studies were performed to evaluate the functional relevance of endogenous angtiotensin II (Ang II) in this model. As shown in Figure 4, AT_1 receptor blockade significantly reduced the polydipsia and polyuria typical of LKRs ($P < 0.05$). These effects were associated with abrogation of glomerular hyperfiltration, as documented by measurements of the clearance of endogenous creatinine, and with a reduction in $U_{NAG}V$. $U_{Na}V$ and FE_{NA} were increased by AT_1 receptor blockade in LKR (from 0.50 ± 0.17 to 1.01 ± 0.07 mmol/day and from 0.07 ± 0.01 to 0.21 ± 0.03%, $P < 0.01$ for both comparisons), whereas these parameters remained unchanged in controls (from 0.34 ± 0.07 to 0.31 ± 0.07 mmol/day and from 0.09 ± 0.01 to 0.09 ± 0.01%, P = NS). The difference in $U_{Kall}V$ between strains remained unchanged following Ang II antagonism.

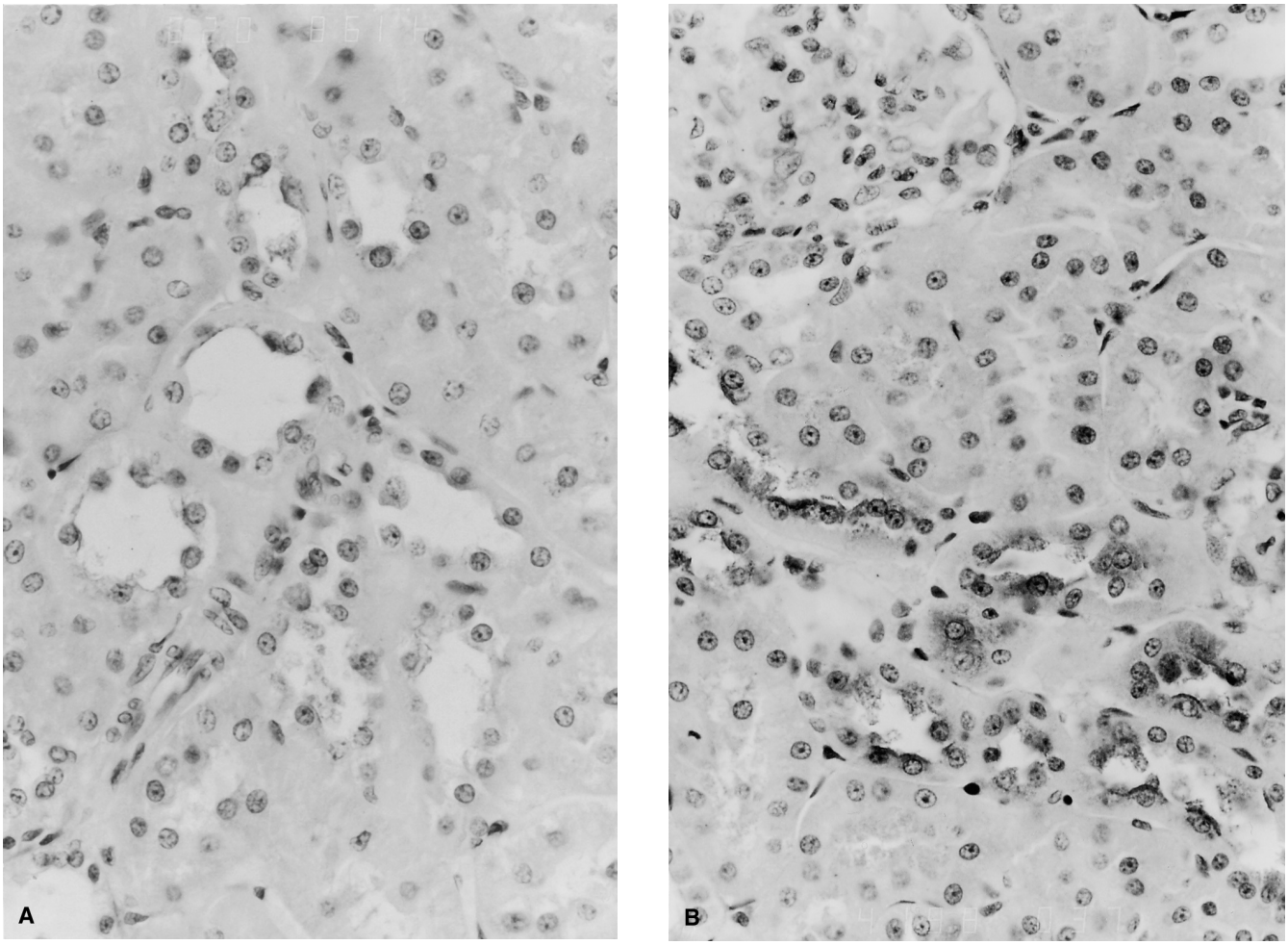


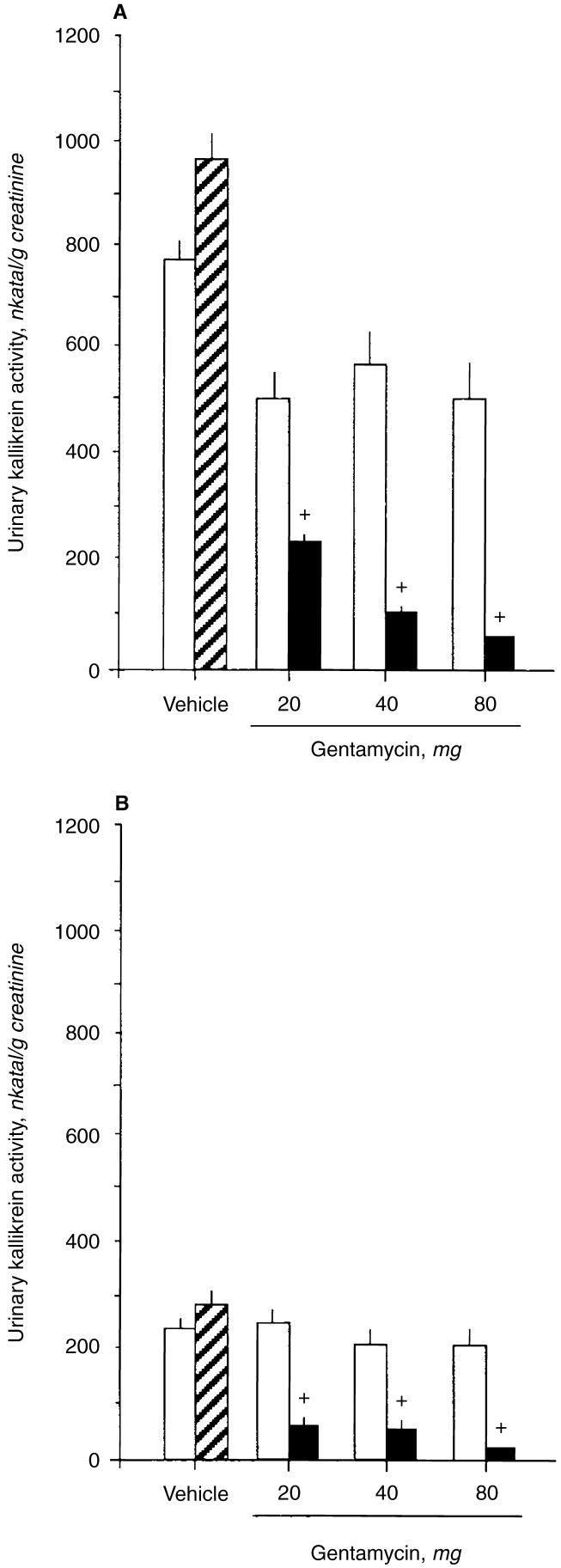
Fig. 2. Histologic sections of kidneys from Wistar rat and low kallikrein rat (LKR), stained with antibody against rat kallikrein (×120 magnifications). Control Wistar rat (*B*) illustrating typical normal staining of distal tubular cells positive for kallikrein (arrow). (*A*) LKRs illustrating the faint staining of tubular cells (arrow); tubular dilation is also shown.

Table 2. Effects of gentamycin on renal function and urinary kallikrein excretion

	Wistar rats				LKR			
Gentamycin *mg*	0	20	40	80	0	20	40	80
Body weight *g*	321 ± 9	362 ± 30	324 ± 19	291 ± 21[a]	338 ± 10	368 ± 25	351 ± 25	309 ± 23[a]
UV *mL/day*	21 ± 7	28 ± 4	23 ± 4	31 ± 5	61 ± 4[b]	55 ± 3[b]	44 ± 4[b]	54 ± 12[b]
GFR *mL/min/g*	0.76 ± 0.04	0.67 ± 0.08	0.57 ± 0.07	0.62 ± 0.06	1.49 ± 0.03[b]	0.95 ± 0.05[bc]	0.86 ± 0.08[bc]	0.73 ± 0.1[c]
$U_{Na}V$ *mmol/day*	0.46 ± 0.14	0.59 ± 0.13	0.51 ± 0.12	0.33 ± 0.17	0.56 ± 0.20	0.92 ± 0.11	0.94 ± 0.14	0.46 ± 0.11
FE_{Na} *%*	0.13 ± 0.02	0.16 ± 0.20	0.15 ± 0.02	0.08 ± 0.01	0.08 ± 0.01[b]	0.15 ± 0.02[c]	0.19 ± 0.02[c]	0.09 ± 0.01
U_{Kall} V *nkaal/g cr*	942 ± 71	223 ± 54[c]	105 ± 35[c]	51 ± 11[c]	318 ± 54[b]	57 ± 8[bc]	51 ± 10[bc]	26 ± 10[bc]
$U_{NAG}V$ *U/day*	233 ± 61	837 ± 70[c]	969 ± 214[c]	816 ± 290[c]	370 ± 68[b]	284 ± 28[b]	684 ± 76[c]	826 ± 204[c]
Hematocrit *%*	44.9 ± 1.4	49.5 ± 2.4	45.0 ± 3.3	54.7 ± 3.3[c]	60.3 ± 2.2[b]	52.0 ± 1.4[c]	49.7 ± 1.3[c]	53.7 ± 2.4[c]
Kidney weight *g/100 g*	0.36 ± 0.02	0.38 ± 0.01	0.46 ± 0.03[c]	0.56 ± 0.06[c]	0.39 ± 0.01	0.42 ± 0.01	0.41 ± 0.02	0.58 ± 0.05[c]

Values are mean ± SEM. Abbreviations are in Table 1 and: UV, urinary volume; GFR, glomerular filtration rate; $U_{Na}V$, urinary sodium volume; FE_{Na}, fractional excretion of sodium; $U_{Kall}V$, urinary kallikrein volume; $U_{NAG}V$, urinary N-acetyl-β-D-glucosaminidase volume.

$P < 0.05$: [a] vs. Basal, [b] vs. Wistar rats, [c] vs. Vehicle



As shown in Figure 5 A–C, acute blockade of the AT_1 receptor resulted in a more pronounced blood pressure-lowering effect in LKRs ($P < 0.05$). In addition, renal vasodilation was observed in both groups, with this effect occurring more promptly in LKRs.

As shown in Figure 5 D–F, acute volume expansion caused a modest decrease in MBP in controls ($P < 0.05$), but not in LKRs. In controls, a decrease in renal vascular resistance (RVR) occurred during the first six minutes following volume expansion ($P < 0.05$), whereas no significant change was detected in LKRs (P = NS).

Renal concentration ability

As shown in Figure 6, water deprivation revealed the integrity of the concentration mechanisms in both strains.

DISCUSSION

Genetically engineered animal models have been generated that lack single components of the kallikrein-kinin system. Kininogen-deficient rats and kallikrein or kinin B_2 receptor knockout mice exhibit distinct cardiovascular alterations, namely blood pressure sensitivity to salt [28] and dilative left ventricular remodeling with aging (abstract; Meneton et al, *Hypertension* 34:333, 1999) [24]. Information gained from this strategy has widened our knowledge on the physiological relevance of the kallikrein-kinin system in cardiovascular function. However, the term "model" should be cautiously used to denote similarity with human disease states, inasmuch as a total deficiency of any component of the kallikrein-kinin system has never been described in humans. Furthermore, indiscriminate abrogation of expression achieved by gene disruption in a germline substantially differs from the defect observed in hypertensive patients, which consists of a reduced urinary output of the enzyme. In this context, the LKR strain represents a more appropriate model to reproduce the functional impact than an inherited defect in renal kallikrein might have in humans.

In LKRs, kallikrein expression was not universally depressed in all tissues examined: Enzyme activity was reduced at kidney level, increased in the heart, and unchanged in the pancreas, liver, and submandibulary glands. Within the kidney, kallikrein immunoreactivity was restricted to the distal tubule, a result consistent with other immunocytochemistry reports [2]. Using immunoistochemical analysis, we have documented a reduced numerical density of kallikrein-positive tubular cells in renal sections of LKR. However, at variance with our earlier studies [22], the faint immunoreactive signal

Fig. 3. Urinary kallikrein activity in normal Wistar rats (*A*) and LKR (*B*) under basal conditions (□) and after gentamycin (■) or vehicle (▨). Gentamycin administration reduced urinary kallikrein in both strains. Data are means ± SEM ($N = 6$ each group). $^{+}P < 0.05$ vs. basal.

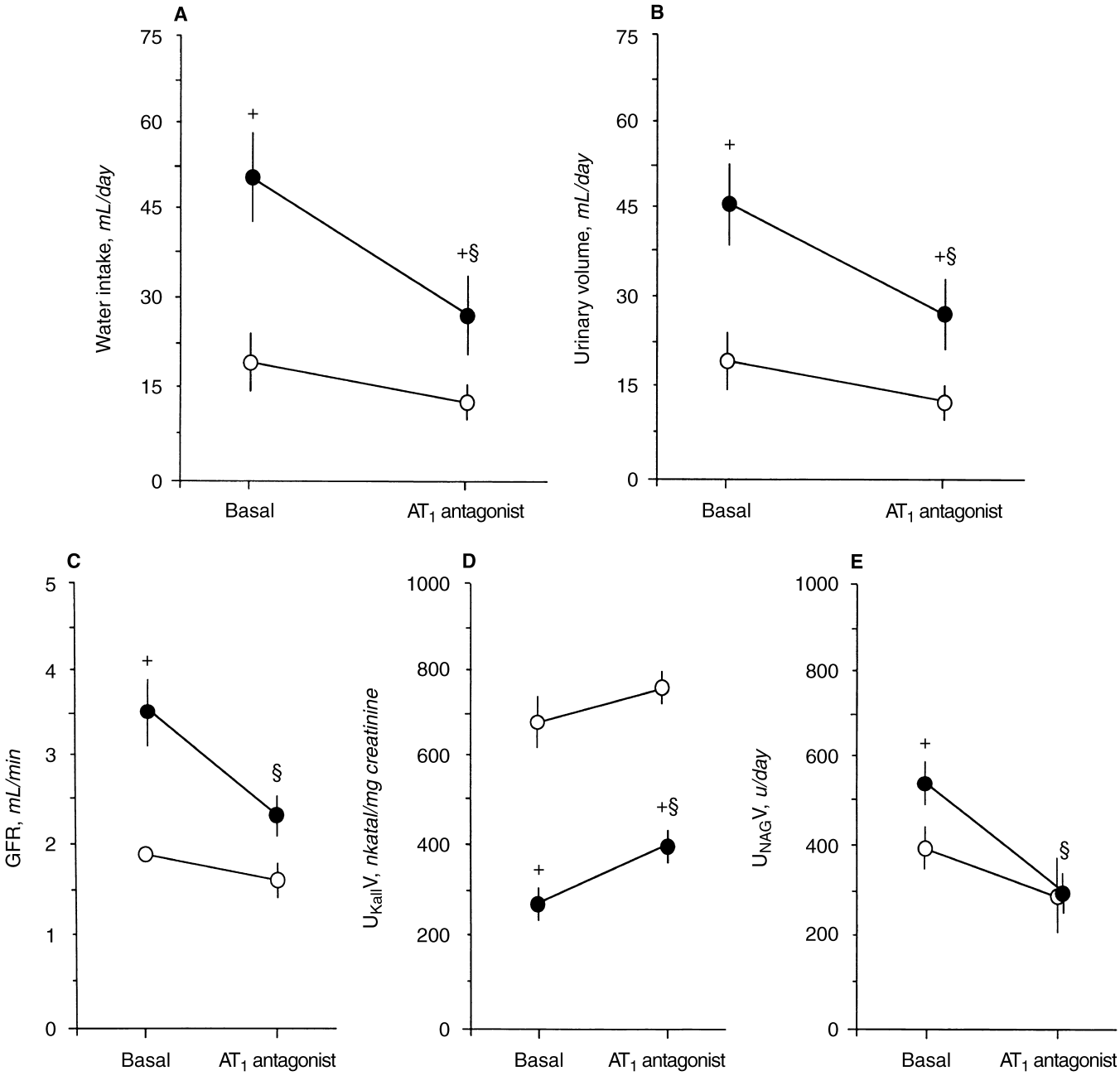
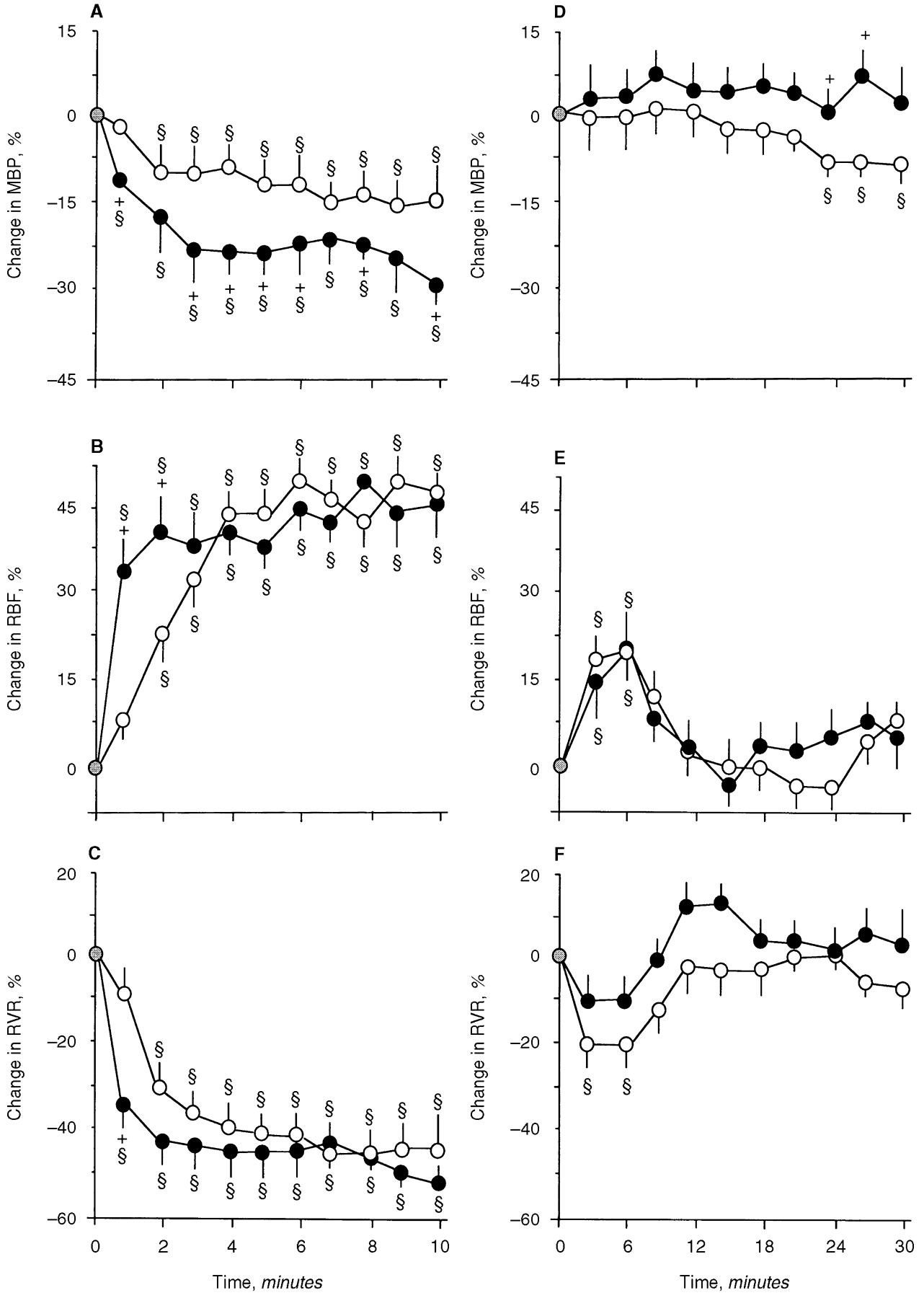


Fig. 4. Effect of angiotensin II subtype 1 (AT_1) receptor blockade on renal function in normal Wistar rats (○) or LKR (●). LKR showed polydipsia, polyuria, glomerular hyperfiltration, and increased enzymuria under basal conditions, with these alterations being partially corrected by AT_1 receptor blockade. In addition, fractional sodium excretion was increased by AT_1 receptor blockade in LKRs (data shown in the **Results** section). Data are means ± SEM ($N = 6$ each group). $^{+}P < 0.05$ vs. control; §$P < 0.05$ vs. basal.

detected at the level of distal tubule of LKR was not associated with any increase of staining in proximal tubules. This discrepancy may be explained with the fact that in contrast to the antiserum used earlier [22], the antibody employed here does not cross-react with esterase A. It remains unknown whether an increase in esterase A, a member of kallikrein multigene family, may represent a compensatory response to the reduction in renal kallikrein synthesis.

Blood pressure is reportedly increased in conscious LKR [22]. Our current study consistently shows increased MBP levels in LKRs under the effects of anesthesia. In the same animals, myocardial contractility was apparently depressed as indicated by reduced dP/dt_{max}. However, the bradycardia observed in these animals may have influenced the MBP parameter. Further studies are necessary to address the possible development of myocardial remodeling and dysfunction in LKRs, and


A
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Change in MBP, %
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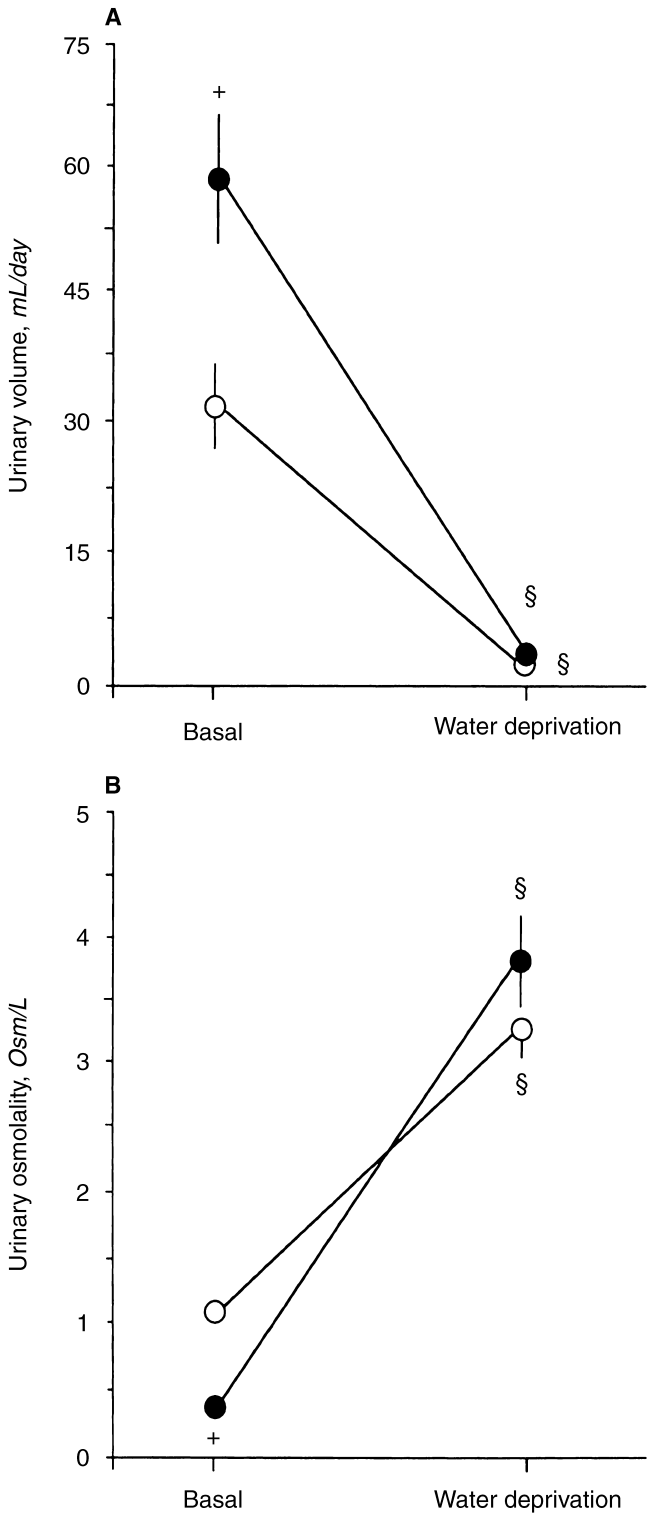

Fig. 6. Effects of water deprivation on UV (*A*) and osmolality (*B*) in normal Wistar rats (○) or LKR (●). Water deprivation revealed intact urinary concentrating mechanisms in LKR. Data are means ± SEM. $^{+}P < 0.05$ vs. control; §$P < 0.05$ vs. basal.

to recognize the relevance of the differential kallikrein expression pattern in the heart and kidney.

Compared our previous studies [22], we found that further inbreeding of the LKR results in additional phenotypical features, namely polydipsia, polyuria, and increased GFR. Polyuria apparently occurs in spite of intact urinary concentrating mechanism, suggesting that the antidiuretic hormone (ADH) system is functioning properly in these rats. Since the high urinary output was not affected by maneuvers that decrease GFR, that is, gentamicin nephrotoxicity, it appears unlikely that polyuria is the consequence of a primary impairment in glomerulotubular balance. Instead, it may be that these rats have a central disturbance in water drinking.

The present data, especially those regarding baseline hematocrit and poor weight gain, suggest the presence of extracellular fluid volume contraction and hemoconcentration in the LKR. Under these conditions, one could envision that preservation of GFR may be possible only as a consequence of activating counter-regulatory systems. We hypothesized that a defective renal kallikrein activity might leave Ang II unbalanced at the glomerular and tubular levels. In the present study, PRA was found to be reduced in LKR. This result, unexpected on the basis of postulated extracellular fluid volume contraction, may be compatible with the assumption that kallikrein represents one of the pro-renin activating enzymes [29]. We also documented that the functional renal responses to antagonism of endogenous Ang II were enhanced in these rats. In fact, glomerular hyperfiltration was normalized, and sodium excretion was significantly increased by chronic blockade of AT_1 receptors. Enhanced renal vasodilation observed in LKRs in response to acute AT_1 receptor blockade also favors our theory. Furthermore, altered renal hemodynamic response to acute volume expansion in LKR resembles that observed in animals with Ang II-mediated hypertension [27]. All together, these results suggest that in the presence of defective kallikrein activity, the kidney becomes more sensitive to endogenous Ang II. It is noteworthy to remember that urinary kinin excretion is reportedly normal in LKRs [22]. Although infusion of kallikrein corrects the renal abnormalities, this action may be independent of

Fig. 5. Effect of acute AT_1 receptor blockade (A–C) or intravenous volume loading (D–F) on systemic and renal hemodynamics in normal Wistar rats (●) or LKR (○). For the AT_1 blockade, LKRs showed a greater reduction in mean blood pressure (MBP) and a more rapid renal vasodilation compared with controls ($N = 10$ each group). For volume loading, LKR exhibited blunted systemic and renal vasodilatory response compared with controls ($N = 7$ each group). Data are means ± SEM. $^{+}P < 0.05$ vs. control; §$P < 0.05$ vs. basal.

the kininogenase activity of kallikrein. Therefore, further characterization of this rat model is required before any conclusions can be made about the involvement of kinins in any functional changes. It would be worthwhile also to explore whether the hyperfiltration observed in LKR exerts long-term consequences. It is tempting to predict that this condition may favor renal functional deterioration with aging.

Finally, we investigated the possibility that the low-kallikrein phenotype may represent a marker of gentamycin nephrotoxicity. This is an important issue since nephrotoxicity occurs in some 30% patients treated with gentamycin and represents a limiting factor for clinical usage. In the absence of pre-existing renal abnormalities, no biochemical test is available to predict the development of renal damage. Gentamycin administration decreased urinary kallikrein excretion in both strains and caused similar functional and histologic alterations indicative of tubular damage. However, a marked decrease in GFR was observed only in LKR. A reduction in GFR is a relatively late manifestation of aminoglycoside nephrotoxicity, and its rapid occurrence in LKR indicates a particular sensitivity of glomerular regulatory mechanisms to gentamycin-induced toxicity.

Altogether, the distinctive functional alterations seen in LKRs support the notion that the kallikrein-kinin system can counteract the vasoconstrictor, sodium-retaining action of Ang II. In perspective, LKRs represent a useful model to study the impact that environmental factors may have on cardiovascular and renal function in the context of an impaired renal kallikrein activity.

ACKNOWLEDGMENT

This work was supported by a 60% grant from the Minister of Universities and Scientific and Technological Research (MURST).

Reprint requests to Paolo Madeddu, M.D., Gene Therapy Section, Istituto Nazionale Biostrutture e Biosistemi, 07033 Osilo (Sassari), Italy. E-mail: madeddu@yahoo.com

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